Amendments to the Specification:

Please amend the specification as follows:

Please replace paragraph starting at page 1, line 4, with the following rewritten paragraph:

The present application is a division of U.S. Patent Application Serial No. 09/476,484, filed December 30, 1999, which claims priority to U.S. Provisional Patent Application Serial No. 60/114,465 ~~by Schlessinger, Okigaki, and Gishizky, entitled PYK2 and Inflammation~~, filed December 30, 1998 ~~(Lyon & Lyon Docket No. 236/075)~~ which ~~is~~ are hereby incorporated by reference herein in their ~~its~~ entirety, including any drawings, tables, or figures.

The present application is related to U.S. application Serial No. 08/357,642 (now U.S. Patent No. 5,837,524), ~~by Lev and Schlessinger, Lyon & Lyon Docket No. 209/070, entitled "PYK2 related Products and Methods",~~ filed December 15, 1994; Serial No. 08/460,626 (now U.S. Patent No. 5,837,815), ~~by Lev and Schlessinger, Lyon & Lyon Docket No. 211/121, entitled "PYK2 related Products and Methods",~~ filed June 2, 1995; and Serial No. 08/987,689 ~~by Lev and Schlessinger, Lyon & Lyon Docket No. 230/110, entitled "PYK2 related Products and Methods",~~ filed December 9, 1997, all of which are hereby incorporated by reference herein in their entirety including any drawings, figures, or tables.

Please replace paragraph starting at page 22, line 5, with the following rewritten paragraph:

Figures 4a~~,~~ and 4b~~, and 4c~~ demonstrate Carageenen induced cellular infiltration in murine air pouches. ~~Fig. 4a shows tissues from wild type +/+ and *pyk2-/-* air pouches that were treated with Carageenen for 10 hours after injection. Samples were formalin-fixed and paraffin-embedded. Sections were stained with Hematoxylin and Eosin.~~ Figure ~~4b~~ 4a shows a number of cells infiltrating into wild type +/+ and *pyk2-/-* air pouches 10 hours after injection with Carageenen. Figure ~~4c~~ 4b shows a fraction of cells infiltrating into wild-type +/+ and *pyk2-/-* mice air pouches 10 hours after injection with Carageenen.

Please replace paragraph starting at page 22, line 13, with the following rewritten paragraph:

Please replace paragraph starting at page 22, line 13, with the following rewritten paragraph:

~~Figures 5a and 5b~~ Figure 5 ~~demonstrate~~ demonstrates influenza virus-induced inflammation. ~~Fig. 5a shows histologic sections that were made of the lung from influenza virus-infected wild-type +/+ and *pyk2-/-* mice 4 days after infection with the virus. Lungs were formalin-fixed and paraffin-embedded. Sections were stained with Hematoxylin and Eosin.~~ Fig. ~~5b~~ 5 shows the time course of the survival of mice at different doses of influenza virus.

Please delete paragraph starting at page 23, line 15:

~~Figures 9a, and 9b show morphological abnormalities and impaired cell migration in PYK2-/- macrophages. Figure 9a shows PYK2-/- (b, d, f) or wild type macrophages (a, c, e) were plated on tissue culture dishes. Micrographs of unstimulated (a, b) or SDF1a-stimulated macrophages (c, d, e, f). White arrows mark long multidirectional processes and white broken arrows mark multidirectional lamellipodia that are seen in PYK2-/- but not in wild type macrophages. Figure 9b shows micrographs of SDF-stimulated or unstimulated PYK2-/- or wild type macrophages plated on tissue culture dishes at different time points. White arrows mark the original point of cell movement, large black arrows mark cell contraction and small black arrows mark lamellipodia.~~

Please replace paragraph starting at page 23, line 25, and bridging page 24, with the following rewritten paragraph:

Figures ~~10a and 10b~~ 9a and 9b show measurement of contractile capacity of lamellipodia in wild type or PYK2-/- macrophages by laser tweezers. Figure ~~10a~~ 9a shows plots of bead displacement from leading edge as a function of time. Fibronectin coated beads were positioned with tweezers on the lamellipodia of PYK2-/- or wild type macrophages of unstimulated (top panels) or MIP1α-stimulated (bottom panels) cells near the leading edge at time 0. The trap remained on for approximately 60 sec. as indicated by shaded area. Two upper plots present beads displacement on non-stimulated macrophages and two lower plots present displacement on MIPlα-stimulated macrophages. Figure ~~10b~~ 9b shows a histogram of

ratio (%) of beads displaying escape from trapped field by laser tweezer. All the beads on the lamellipodia of PYK2-/- or wild type macrophages before and after stimulation by MIP1α were subjected to a restraining force after initial bead-cell contact for 60 sec. Ratios of beads which escaped and moved rearward were scored. Left column shows score of non-stimulated macrophages and right column shows score of MIP1α-stimulated macrophages.

Please delete paragraph starting at page 24, line 10:

~~Figures 11a, 11b, 11c, and 11d show changes in cytoskeletal organization in PYK2-/- macrophages. PYK2-/- and wild type macrophage were plated on tissue culture dishes, fixed by 4% paraformaldehyde and stained by fluorescently labeled phalloidin (A and B) or anti-α-tubulin antibodies (D). In Figure 11a white arrows in (b) and (d) indicate long multi-directional processes and broken white arrows in (b) and (d) indicate membrane ruffles in PYK2-/- macrophages. In Figure 11b, top and side view of F-actin distribution were visualized with a confocal microscope. Arrowheads indicate reorganized F-actin in PYK2-/- macrophages. Black arrows reveal the planes of the slices generated in top and side views. In Figure 11c, PYK2-/- and wild type macrophages were placed in MIP1α gradient concentration for 60 min., fixed and then stained with fluorescently labeled phalloidin. The top left part of the field was exposed to the highest concentration of MIP1α. White arrows mark regions with strong phalloidin labeling. In Figure 11d, nonstimulated (a, c) or MIPα-stimulated (b, d) macrophages. White arrowheads in (b) and (d) demonstrate microtubules assembled at cell periphery in PYK2-/- macrophages. White arrow in (d) demonstrates decreased intensity of MTOC in PYK2-/- macrophages.~~

Please replace paragraph starting at page 24, line 26, and bridging page 25, with the following rewritten paragraph:

Figures ~~12a, 12b, 12c, and 12d~~ 10a, 10b and 10c show a comparison of cell signaling in wild type and PYK2-/- deficient macrophages. For Figure 12a, PYK2-/- and wild type macrophages were plated on fibronectin coated dishes for 0.5, 1 and 2 hour, then lysed and incubated with GST-RBD(rho-binding domain) bound to gluthatione beads as described in material and methods. The amount of rhoA: GTP complex was determined by

immunoblotting with anti-rhoA antibodies. ~~For Figure 12b, the rho inhibitor C3 was microinjected together with fluorescently labeled dextran into wild type macrophages. After three hours incubation, the morphology of microinjected or non-injected cells was compared by Nomarsky microscopy (right). The injected macrophages were identified by their fluorescence (left). Arrowheads mark microinjected macrophages, arrows mark lamellipodia and broken arrows mark long process.~~ Figure ~~12c~~ 10b shows Ca^{+2} release in PYK2-/- or wild type macrophages. Macrophages loaded by fura-2 were stimulated by MIP1α in Ringer's solution with 2mM calcium. Changes in fluorescence intensity as a function of time were traced in the fura-2 loaded cells following stimulation with MIP1α or ATP. Figure ~~12d~~ 10c shows production of Ins(1,4,5)P_3 in wild type or PYK2-/- macrophages. Wild type or PYK2-/- macrophages were labeled with myo-[^{3}H]inositol for 24 hours. After MIPlα stimulation the lipid fraction was extracted and analyzed by HPLC. Closed square indicate production of inositol (1,4,5) triphosphate in wild type macrophages and open circle indicate production of inositol (1,4,5) triphosphate in PYK2-/- macrophages. The experiment was done in duplicates and repeated three times.

Please replace paragraph at page 25, line 17, before the Detailed Description of the Invention, with the following rewritten paragraph:

Figures ~~13a and 13b~~ 11a and 11b show data related to myelin oligodendrocyte glycoprotein induced experimental autoimmune encephalomyelitis (MOG-induced EAE). Figure ~~13a~~ 11a shows that both wild type and PYK2 -/- mice were susceptible to MOG-induced EAE. Figure ~~13b~~ 11b shows that the proliferative response toward MOG was delayed in T-cells from draining lymph nodes from PYK2 -/- mice as compared to T-cells from wild type mice.

Please replace the first paragraph on page 47 with the following rewritten paragraph:

A DNA fragment of λ900 bp corresponding to residues 362-647 of PYK2 was amplified by PCR utilizing he following oligonucleotide primers **(SEQ ID NO: 1)** 5'-CGGGATCCTCATCATCCATCCTAGGAAAGA-3' (sense) and **(SEQ ID NO: 2)** 5'-CGGGAATTCGTCGTAGTCCCAGCAGCGGGT-3' (antisense).

Please replace the second paragraph on page 47 with the following rewritten paragraph:

The influenza virus hemagglutinin peptide **(SEQ ID NO: 3)**(YPYDVPDYAS) was fused to the C-terminus of PYK2 utilizing the following oligonucleotide primers in the PCR reaction: **(SEQ ID NO: 4)** 5'-CACAATGTCTTCAAACGCCAC-3' and **(SEQ ID NO: 5)** 5-GGCTCTAGATCACGATGCGTAGTCAGGGACATCGTATGGGRACTCTGCAGGTGG GTGGGGCCAG-3'. The amplified fragment was digested with RsrII and Xbal and was substituted with the corresponding fragment of PYK2. The nucleotide sequence of the final construct was confirmed by DNA sequencing.

Please replace the fifth paragraph on page 47 with the following rewritten paragraph:

In order to construct a kinase negative mutant, Lys (457) was substituted to Ala by site directed mutagenesis utilizing the 'Transformer Site-Directed mutagenesis Kit' (Clontech). The oligonucleotide sequence was designed to create a new restriction site of Nrul. The nucleotide sequence of the mutant was confirmed by DNA sequencing. The oligonucleotide sequence that was **(SEQ ID NO: 6)** used for mutagenesis was: 5'-

Please replace paragraph starting at page 55, line 1, with the following rewritten paragraph:

Ten hours after carrageenan injection into wild type or PYK2-/- mice, tissue sections of the injected lesion were examined microscopically for the presence of infiltrating macrophages and neutrophils ~~(Fig. 4a)~~. Tissues from wild type +/+ and *pyk2-/-* air pouches were treated with Carageenen for 10 hours after injection. Samples were formalin-fixed and paraffin-embedded. Sections were stained with Hematoxylin and Eosin. The average number

of infiltrating cells in wild type mice was $4.8x10^6$ per injected area, while in the PYK2-/- mice there was an average of only $2.8x10^6$ cells per injected area (Fig. ~~4b~~ 4a). Morphological examination of the infiltrating cells indicated that macrophages comprised approximately 70% of the infiltrate in wild-type mice but only 20% of the infiltrate in the PYK2-/- mice, the remaining cells were primarily neutrophils (Fig. 4b). These data show that the failure of PYK2-/- macrophages to migrate effectively *in vitro* is correlated with a striking deficit in inflammatory infiltration *in vivo*.

Please replace paragraph starting at page 55, line 15, with the following rewritten paragraph:

Influenza virus-induced inflammation in mice lungs was studied in lung sections from influenza virus-infected wild-type +/+ and Pyk2-/- mice 4 days after infection with the virus. Lungs were formalin-fixed and paraffin-embedded. Sections were stained with Hematoxylin and Eosin ~~(Fig. 5a)~~. Histologic sections were made of the lung from influenza virus-infected wild-type +/+ and *pyk2-/-* mice 4 days after infection with the virus. Figure ~~5b~~ 5 shows a time course of the survival of mice at different doses of influenza virus.

Please replace paragraph starting at page 57, line 14, with the following rewritten paragraph:

Macrophages normally express high levels of PYK2 and bearly detectable levels of FAK (Lipsky, *et al.* (1998) *J. Biol. Chem.* 273:11709-11713). It is possible that these cells might be more susceptible to the loss of PYK2. We have first compared the morphology of peritoneal macrophages from wild type or PYK2-/- mice 30 minutes after plating these cells on coverslips or on tissue-culture dishes. The ~~micrograph~~ micrographs ~~depicted in Fig. 9 (panel a) shows~~ showed that after adhesion wild-type macrophages adopt a typical round shape. Treatment of wild type macrophages with the chemokine SDF1α resulted in rapid induction of lamellipodia and enhancement of cell spreading ~~(Fig 9a, panel c and e)~~. In contrast, most PYK2-/- macrophages displayed a flattened morphology with extensive membrane spreading even without chemokine treatment ~~(Fig. 9a, panel b)~~. Addition of

SDF1α further increased the formation of pseudopodia as well as the appearance of long processes ~~(Fig. 9a, panel d and f)~~.

Please insert the following paragraph on page 57, at line 26, before the start of the last paragraph beginning at line 26:

Morphological abnormalities and impaired cell migration in PYK2-/- macrophages were observed. PYK2-/- or wild type macrophages were plated on tissue culture dishes. Micrographs of unstimulated or SDF1a-stimulated macrophages were obtained and long multidirectional processes were observed. Multidirectional lamellipodia were seen in PYK2-/- but not in wild type macrophages. Micrographs of SDF-stimulated or unstimulated PYK2-/- or wild type macrophages plated on tissue culture dishes at different time points were obtained. The original point of cell movement was observed. Cell contraction and small lamellipodia were observed.

Please replace paragraph starting at page 57, line 26, and bridging page 58, with the following rewritten paragraph:

In view of the enhanced spreading with multiple pseudopodia and long processes as well as the enhanced substrate attachment displayed by PYK2-/-macrophages, we considered that macrophage migration in response to chemotactic stimulation could be impaired. We treated wild type and PYK2-/- macrophages with SDF1α and observed cell morphology and movement at ten minute intervals following chemokine stimulation ~~(Fig. 9b)~~. This experiment shows that within ten minutes wild-type macrophages become polarized, developing lamellipodia, on one side of the cell. At later time points (>20 minutes), the cell body moves in the direction established by the leading edge, detaching from the substrate at the trailing edge. In contrast, formation of new lamellipodia by PYK2-/- macrophages in response to SDF1α, was delayed as compared to wild-type cells ~~(Fig. 9b)~~. Furthermore, the cell body showed reduced ability to follow the leading edge and failed to detach from the substratum. Over time, the PYK2-/- macrophages extended lamellipodia in several directions with similar failure to detach from the substrate. Eventually, most PYK2-/- macrophages developed several pseudopodia-like processes with minimal net migration. Overall, PYK2-/-

macrophages are able to form a leading edge in response to a chemotactic stimulus, albeit with slower kinetics. However, these cells are unable to move the cell body after the leading edge efficiently, and fail to detach the lagging edge from the substratum.

Please replace paragraph starting at page 58, line 18, and bridging page 59, with the following rewritten paragraph:

Microscopic observation of migrating macrophages revealed that PYK2-/- cells could extend lamellipodia but the cell body failed to flow into the newly formed leading edge. We suggested that the contractile activity of the cytoskelton in the lamellipodia was impaired in PYK2-/- macrophages. The contractile force was determined by measuring the rearward movement toward the nucleus of beads coated with recombinant fragment of fibronectin (FN type III domains 7-10) on lamellipodia in opposition to an immobilizing force generated by optical tweezers (Choquet, *et al.* (1997) *Cell 88*:39-48; Felsenfeld, et al. (1999) *Nature Cell Biology 1*:200-206). The velocity of rearward movement of the beads in opposition to this force represents a function of (i) the strength of association between the cytoskeleton and the integrins bound to the fibronectin on the bead, and (ii) the strength of traction force generated by the cytoskeleton itself (Choquet, *et al.* (1997) *Cell 88*:39-48; Sheetz, *et al.* (1998) *Trends Cell Biol. 8:*51-54). Immobilizing force by optical tweezers was applied to the beads on the lamellipodia of the cells and the movement of the bound beads was monitored. Representative plots of the distance of bead displacement versus time in wild type or PYK2-/- macrophages is presented in Fig ~~10a~~ 9a.

Please replace paragraph starting at page 59, line 3, with the following rewritten paragraph:

The beads on the lamellipodia from wild type macrophages exhibited rearward movement and escaped from the force field of the laser trap. After chemokine stimulation, the velocity of bead movement on the lamellipodia of wild type macrophages was increased and the beads escaped more quickly. With the immobilizing force exerted by the optical tweezers, more than 50% of the beads that were attached to the lamellipodia of either stimulated or unstimulated wild type macrophages were able to escape from the force field of

the optical trap. In contrast, beads bound to the lamellipodia of PYK2-/- macrophages did not exhibit rearward movement in presence or absence of chemokine stimulation (Fig. ~~10a~~ 9a). No beads that were attached to the lamellipodia of either stimulated or unstimulated PYK2-/- macrophages were able to escape from the optical trap (Fig. ~~10b~~ 9b). Overall, rearward movement, *e.g.* the contractile force generated by the cytoskeleton, is impaired in PYK2-/- macrophages in comparison to the contractile force generated in wild type macrophages.

Please replace paragraph starting at page 59, line 19, and bridging page 60, with the following rewritten paragraph:

We next examined the status of the cytoskeleton in PYK2-/- macrophages. Visualization of cells stained with fluorescent phalloidin revealed an increase of F-actin in membrane ruffles in PYK2-/- as compared to wild-type macrophages ~~(Fig. 11a)~~. Analysis of F-actin distribution by confocal microscopy revealed an increase in reorganized F-actin underneath the ruffles in PYK2-/- macrophages ~~(Fig 11b)~~. When wild-type macrophages were placed in a chemotactic gradient, phalloidin staining revealed increase in the relative amount of F-actin at the edge of the cell in the region that is exposed to the greatest concentration of chemotactic signal. The distribution of F-actin in stimulated PYK2-/- macrophages was different, in these cells F-actin was distributed at multiple sites along the cell periphery ~~(Fig. 11c, left)~~. In migrating wild-type macrophages F-actin is continuously redistributed towards the leading edge of the cell. This redistribution of F-actin does not occur in PYK2-/- macrophages, probably resulting in the failure of the cells to become properly oriented in a chemotactic gradient.

Please insert the following paragraph on page 60, at line 3, before the start of the first paragraph beginning at line 3:

Changes in cytoskeletal organization were observed in PYK2-/- macrophages. PYK2 -/- and wild type macrophage were plated on tissue culture dishes, fixed by 4% paraformaldehyde and stained by fluorescently labeled phalloidin or anti-α-tubulin antibodies. Long multi-directional processes and membrane ruffles were observed in PYK2-/- macrophages. Top and side view of F-actin distribution were visualized with a confocal

microscope. Reorganized F-actin in PYK2-/- macrophages was observed. The planes of the slices generated in top and side views. PYK2-/- and wild type macrophages were placed in MIP1α gradient concentration for 60 min., fixed and then-stained with fluorescently labeled phalloidin. The top left part of the field was exposed to the highest concentration of MIPlα. Regions with strong phalloidin labeling were observed. Nonstimulated or MIPα-stimulated macrophages were observed. Microtubules assembled at cell periphery in PYK2-/- macrophages were observed. Decreased intensity of MTOC was observed in PYK2-/- macrophages.

Please replace paragraph starting at page 60, line 3, with the following rewritten paragraph:

We have also examined the distribution of microtubules in wild type and PYK2-/- macrophages. It was proposed that microtubules play an important role in driving actin polymerization and leading-edge lamellipodia protrusion through specific rho-GTPases during cell migration (Waterman-Storer, *et al.* (1999) *Nature Cell Biol. 1*:45-50). The organization of microtubules in wild type or PYK2-/- macrophages was visualized by staining permaebilized cells with anti-tubulin antibodies. The results of the experiment ~~depicted in Fig. 11d shows~~ show that the microtubules in PYK2-/- macrophages are more assembled than the microtubules in wild type macrophages. Upon chemokine stimulation, the microtubules of wild type macrophages radiate from the microtubules organizing center (MTOC) while PYK2-/- macrophages display long microtubules that are assembled at the cell periphery into longitudinal directions with decreased intensity towards the MTOC ~~(Fig. 11d)~~. The increased assembly of microtubules in the periphery of PYK2-/- macrophages could be linked to the enhancement in F-actin organization, extensive lamelliopodia formation in this region leading to altered cell polarization.

Please replace paragraph starting at page 60, line 20, and bridging page 61, with the following rewritten paragraph:

The rho family of small G-protein has been implicated in the control of cytoskelal organization leading to changes in cell morphology and cell migration (Ridley, *et al.* (1999)

*Biochem. Soc. Symp. 65:*111-123). It was demonstrated that integrin-induced cell adhesion leads to the activation of rho (Ren, *et al.* (1999) *EMBO J. 18:*578-585). The activated GTP bound form of rho binds to effector proteins that are involved in the control of cytoskeletal organization and contraction of lamellipodia (Allen, *et al.* (1997) *J. Cell Sci. 110:*707-720; Maekawa, *et al.* (1999) *Science 285:*895-898). We have analyzed activation of rho in macrophages by applying a "pull-down" assay using a GST fusion protein containing the binding site from rhotekin for the GTP bound form of rho (Ren, *et al.* (1999) *EMBO J. 18:*578-585). The experiment presented in Fig. 12a shows fibronectininduced activation of rho as a function of time. In contrast, a similar experiment performed with PYK2-/- macrophages revealed reduced activation of rho in the mutant macrophages in response to integrin-induced cell adhesion (Fig. ~~12a~~ 10a).

Please insert the following paragraph on page 61, at line 3, before the start of the first paragraph beginning at line 3:

The rho inhibitor C3 was microinjected together with fluorescently labeled dextran into wild type macrophages. After three hours incubation, the morphology of microinjected or non-injected cells was compared by Nomarsky microscopy . The injected macrophages were identified by their fluorescence. Microinjected macrophages, lamellipodia and long process were observed.

Please replace paragraph starting at page 61, line 3, with the following rewritten paragraph:

We have further examined the role played by rho in the control of macrophage morphology by microinjecting into these cells a specific inhibitor of rho designated C3 (Chardin, *et al.* (1989) *EMBO J. 8:*1087-1092) together with fluorescently labeled dextran as a specific marker ~~(Fig. 12b)~~. This experiment demonstrates that wild type macrophages microinjected with C3, showed a rapid and extensive cell spreading with strong ruffling and formation of long processes similar to the morphological changes seen in PYK2-/- macrophages. However, microinjection of C3 into PYK2-/-macrophages did not cause further changes to those seen in untreated PYK2-/-macrophages ~~(Fig. 12b)~~. Taken together,

these experiments show that rho is activated upon adhesion of macrophages and that reduced activation of rho may be responsible for the enhanced spreading, ruffling and formation of long processes in PYK2-/-macrophages.

Please replace paragraph starting at page 61, line 17, with the following rewritten paragraph:

Calcium plays an important role in the control of a variety of intracellular events as well as in the control of cell shape, and cell movement (Lawson and Maxfield, 1995). We therefore measured cytoplasmic calcium release in single cells in response to MIP1αt stimulation by using quantitative fluorescence microscopy of Fura-2 loaded cells. Treatment of wild type macrophages attached to cover slips showed maximum increase in cytoplasmic [Ca^{+2}j concentration at approximately 300 nM of MIP1α. By contrast, PYK2-/- adherent macrophages did not show an obvious increase in [Ca^{+2}] concentration (Fig. ~~12e~~ 12b). This experiment shows that PYK2 plays an important role in the control ofMIP1α-induced Ca^{+2} release in adherent macrophages. Defect in calcium release may contribute towards the failure of the cells to detach at the rear end leading to impairment in cell migration since proteins that regulate the degradation of focal contact components and disassembly of F-actin require calcium for their action (Witke, *et al.* (1995) *Cell 81:*41-51; Kulkarni, et al. (1999) *J. Biol Chem. 274:*21265-21275).

Please replace paragraph starting at page 61, line 30, and bridging page 62, with the following rewritten paragraph:

A significant proportion of intracellular Ca^{+2} released in response to extracellular signals is mediated by inositol (1,4,5) triphosphate [Ins(1,4,5)P_3] production (Furuichi, *et al.* (1989) *Nature 342:*32-38). We therefore analyzed the production of Ins(1 ,4,5)P_3 in these cells. In this experiment wild type or PYK2-/-macrophages were labeled with [^{3}H] myo-inositol and then stimulated with MIP1α. At various times after MI1α stimulation the production of Ins(1 ,4,5)P_3 was determined by HPLC analysis (Falasca, *et al.* (1998) *EMBO J. 17:*414-422). Wild type macrophages showed a biphasic production of Ins(1,4,5)P_3 with peaks at 20 sec. and 2 min. after MIPlα stimulation. The experiment presented in Fig. ~~12d~~

12c shows that Ins(1,4,5)P_3 production was severely reduced in PYK2-/- macrophages; the peak at 20 sec post stimulation was reduced by approximately 50% as compared to Ins(1,4,5,)P_3 production in wild type macrophages and no Ins(1,4,5)P_3 was generated after 2 min. of MIP1α stimulation (Fig. ~~12d~~ 12c). We have also detected impairment in the production of glycerophosphoinositol, phosphatidylinositol-3-phosphate and phosphatidylinositol-4-phosphate in PYK2-/- macrophages. These findings suggest that PYK2 deficiency may lead to a more general impairment in phosphatidyl inositol metabolism. We have previously shown that PYK2 forms a complex with a family of phosphatidylinositol transfer proteins designated Nirs both *in vitro* and in living cells (Lev, *et al.* (1999) *Mol. Cell. Biol. 19:*2278-2288). The interaction between PYK2 and the phosphatidylinositol transfer proteins and its absence in PYK2-/- macrophages may be responsible for the impairment in phosphatidylinositol metabolism described.

Please replace paragraph starting at page 62, line 23, and bridging page 63, with the following rewritten paragraph:

Experimental Autoimmune Encephalomyelitis (EAE) is an inflammatory demyelinating disease of the central nervous system (CNS) which exhibits a predominantly mononuclear infiltrate, and is widely used as an animal model of multiple sclerosis (Zamvil and Steinman (1990) *Annu. Rev. Immunol 8:*579-621). Since EAE is largely dependent on the activity of macrophages, we have compared the susceptibility of wild type or PYK2-/- mice to EAE by immunizing the mice with Myelin Oligodendrocyte Glycoprotein (MOG) (Johns, *et al.* (1995) *J. Immunol. 154:*5536-5541; Mendel, *et al.* (1995) *Eur. J. Immunol. 25:*1951-1959) and monitoring EAE progression. EAE was induced in PYK2-/- and wild type mice (129Sv) and the clinical course of the disease was monitored daily. As shown in figure ~~13a~~ 11a, both wild type and PYK2-/- mice are susceptible to MOG-induced EAE. However, the onset of EAE was delayed by approximately two days in PYK2 deficient mice as compared to the onset of the disease in wild-type mice. In several experiments PYK2-/- mice showed lower incidence of EAE; while not more than 75% of the PYK2-/- mice came down with the disease, virtually all wild-type mice became sick. However, the outcome of the disease was more severe in PYK2-/- mice, as compared to wild-type mice. For example, in the experiment depicted in Fig. ~~13~~ 11, 50% of PYK2-/- mice (4 of 8) died whereas only

one of 8 wild-type mice succumbed, the remaining mice showed partial or complete clinical recovery. EAE recovery may not be due entirely to a reduction in the proinflammatory stimulus. There is evidence that cytokines and regulatory cells are actively involved in the clinical improvement (Welch, *et al.* (1980) *J. Immunol. 125:*186-189; Karpus, *et al.* (1991) *J. Immunol. 146:*1163-1168; Kennedy, *et al.* (1992) *J. Immunol. 149:*2496-2505); this process appears to be impaired in PYK2-/- mice.

Please replace paragraph starting at page 63, line 15, with the following rewritten paragraph:

Next, draining lymphnode and central nervous system samples were prepared from wild type or PYK2 deficient mice at different times post induction. We have shown that T cells from wild type and mutant mice proliferate equally well in response to anti-CD3 stimulation at all time-points. However, the experiment presented in Fig. ~~13b~~ 11b shows that the proliferative response towards MOG was delayed in T cells from draining lymphnodes from Pyk2-/- mice as compared to T cells from wild-type mice.